Chronic renal denervation increases renal tubular response to P2X receptor agonists in rats: implication for renal sympathetic nerve ablation

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Abstract

Background. Kidney noradrenergic innervation regulates tubular function. Adenosine triphosphate (ATP)—a co-transmitter of norepinephrine—acts on purinoreceptors, including ion channel receptor, P2X. P2X receptor agonists, α,β-methylene ATP (α,β-meATP) and β,γ-methylene ATP (β,γ-meATP), induce natriuresis. Regarding the functional co-localization of adrenoceptors and P2X receptors, we evaluated rat renal tubular system sensitivity to natriuretic action of P2X receptor agonists in chronically denervated kidney.

Methods. Clearance studies with α,β-meATP and β,γ-meATP (intravenous infusion rate, 2 µmol/kg + 20 nmol/kg/min) were performed after bilateral surgical kidney denervation (DNx) and sham-operation (Sham). Na/K-ATPase activity was measured in isolated rat proximal tubules.

Results. In DNx compared with Sham, saline infusion significantly increased renal sodium and urine excretion and P2X receptor agonist infusion was significantly more natriuretic and diuretic. In DNx and Sham, respectively, α,β-meATP increased fractional excretion of sodium (FENa) by 2 ± 0.3 and 0.6 ± 0.1% and urine (FEV) by 1.6 ± 0.3 and 0.9 ± 0.2%; β,γ-meATP had similar effects. In both groups of rats, natriuretic and diuretic actions were abolished by P2 receptor blocker (pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate, PPADS), mean arterial blood pressure and glomerular filtration rate remained unchanged during infusion of P2X receptor agonists and antagonist and basal Na/K-ATPase activities in isolated proximal tubules were similar. Both α,β-meATP and β,γ-me-ATP decreased the Na/K-ATPase activity, with 20% inhibition (P < 0.05) in denervated and innervated rats; these inhibitory effects were abolished in the presence of PPADS.

Conclusions. Decreased renal sympathetic activity enhances the natriuretic effect of P2X receptor stimulation. This effect is probably not related to altered Na/K-ATPase activity in renal proximal tubules.

Keywords: ablation; denervation; natriuresis; P2X receptors

Introduction

It has been demonstrated that hyperactivity of the renal sympathetic nervous system and unwillingness of the kidney to excrete sodium play a role in the pathogenesis of essential hypertension. Renal sympathetic nerves utilize norepinephrine (NE) as a neurotransmitter, affecting the renal arterioles, juxtaglomerular granular cells and tubules of the kidney via adrenergic receptors [1]. In different nerves, NE decreases the renal blood flow (via α1-1-adrenoceptors) and induces both renin secretion (via β1--adrenoceptors) and renal tubular sodium reabsorption (via α1-2-adrenoceptors). In the reverse mode, renal denervation leads to decreased renal tubular sodium reabsorption [2]. Moreover, afferent signalling affects central sympathetic activity and contributes to neurogenic hypertension and renal insufficiency [3, 4]. Consequently, catheter-based renal denervation using low-power radio frequency energy is a strategy for lowering blood pressure [5, 6]. However, most experiments and clinical studies of this procedure have focussed on the adrenergic system and were not explicitly designed to identify and characterize the effects of renal denervation on the actions of other neurotransmitters.

Co-transmitters, including adenosine triphosphate (ATP), are released from sympathetic nerves with NE [7]. Extracellular ATP affects cellular function via P2 receptors, including ion channel receptors (P2X1-7) and metabotropic receptors (P2Y1,2,4,6,11-14), most of which are expressed on the renal vasculature and on the renal epithelium in nephron segments [8]. Activation of P2...
receptors affects renal function [9, 10]. We have recently shown that P2X receptor agonists, α,β-methylene ATP (α,β-meATP) and β,γ-methylene ATP (β,γ-meATP), induce changes in renal haemodynamics and urinary sodium excretion involving renal proximal tubule Na+/K-ATPase [11]. Activation of renal sympathetic neurons using NE and ATP could simultaneously project the action into two targets—adrenergic and purinergic, respectively [12]. Adrenergic and purinergic transmission may affect each other, in turn, modifying renal function. P2 receptors mediate inhibition of NE release [13]. The depletion of nerve activity changes the sensitivity to neuromediators, e.g. denervation of rat vas deferens induces supersensitivity to ATP [14]. However, the relative contribution of each of these neurotransmitters towards influencing renal function is not well established.

From morphological and physiological studies, the question arises of whether there is crosstalk between the adrenergic and purinergic systems in regulating the excretion function of the kidney. The present study addresses this issue by determining the influence of noradrenergic tone provided by the renal nerves on P2X receptor stimulation of tubular function, measured as sodium and water excretion. Our results provide evidence that renal denervation, which is comparable to the catheter-based denervation by low-power radiofrequency, enhances natriuretic effects of P2X receptor activation and that this effect is not related to Na/K-ATPase.

Materials and methods

Animals

The studies were performed on male Wistar rats maintained on a 12-h light/dark cycle. Rats had free access to regular pellet diet (0.25% sodium; Labofeed B, K cynia, Poland) and tap water. On the day of renal denervation, body weights ranged from 225 to 255 g. Experiments were performed on age-matched animals on the 10th day after renal denervation (DNx); sham renal denervation rats were used as controls (Sham).

All experiments were conducted in accordance with a protocol approved by the local Bioethical Committee at the Medical University of Gdansk.

Kidney denervation

Under anaesthesia with thiopental [60 mg/kg intraperitoneally (i.p.); Sandoz, GmbH, Austria], both kidneys were approached retroperitoneally via a flank incision; the renal vessels were stripped of all visible nerves and were painted with a 10% phenol solution. Sham renal denervation consisted of identical anaesthetic and surgical procedures, but the renal nerves were left intact. Before surgery, the animals received gentamycin sulphate (10 mg/kg intramuscular; KRKA d.d., Novo Mesto, Slovenia) injection. Following the denervation and sham procedure, rats recovered from anaesthesia and surgery for 10 days prior to the terminal experiment.

To verify the effectiveness of renal denervation, the NE content in renal tissue homogenate was measured using an enzyme immunoassay kit (Labor Diagnostica Nord, GmbH & Co. KG, Germany). On the 10th day after surgery, NE concentrations (n = 6) were 28 ± 2 (DNx) and 233 ± 19 (Sham) ng/g wet tissue (P < 0.01). ATP concentrations (n = 4) in intestinal fluid were estimated as 4.3 ± 0.7 nM (DNx) and 5.3 ± 0.4 nM (Sham) using a no-net-flux microdialysis technique and luciferin–luciferase-based assay.

Metabolic balance studies

Rats (n = 10) were housed singly in metabolic cages for 48 h before and 10 days after renal denervation (DNx). Animals had free access to a regular pellet diet and drinking water. During the first 24 h, the rats were allowed to stabilize. Urine was collected on the second day; urine volume and urinary electrolyte excretions were measured.

Na/K-ATPase activity in renal proximal tubules

Isolation of the proximal tubules. Under anaesthesia with thiopental (60 mg/kg i.p.), kidneys were removed and sliced. Proximal tubules were isolated from cortex slices using collagenase digestion and Percoll gradient centrifugation [15]. Tubule suspension purity was assessed by measuring enzyme activities of the proximal tubule marker fructose-1,6-bisphosphatase and distal tubule marker hexokinase [16, 17]. Tubular suspension was characterized by a 3.1-fold enrichment of fructose-1,6-bisphosphatase activity and a 3.2-fold impoverishment of hexokinase activity.

Measurement of Na/K-ATPase activity. The proximal tubules—0.3 mg suspended in ice-cold Krebs–Henseleit saline (KHS, pH 7.40), previously gassed with 95% O2/5% CO2 at 37°C—were incubated in the absence (basal) or presence of 10−7 M pyridoxal-phosphate-6-azophenyl-2′,4′-disulphophenyl (PPADS) for 3 min and subsequently with 10−6 M α,β-meATP or β,γ-meATP at 37°C for 15 min (total volume 2 mL). Following incubation, the samples were placed on ice, centrifuged and the tubules permethylized by freezing and thawing in deionized water. The thawed samples were subsequently used for Na/K-ATPase activity measurement [18]. The specific Na/K-ATPase activity was calculated by subtracting the ATPase activity in the presence of ouabain from the total ATPase activity.

Clearance studies

Animal preparation. The rats were anaesthetized with i.p. injection of 60 mg/kg body weight thiopental. The animals were placed on a thermostatically controlled table heated to 37°C. A polyethylene tube was placed in the trachea to ensure a free airway. Polyethylene catheters (PE-50) were placed into the femoral vein for saline infusion and the femoral artery for periodic blood sampling and continuous monitoring of the mean arterial blood pressure (MAP) via Statham transducer. The catheter inserted into the femoral artery was filled with isotonic saline solution containing 30 IU/mL heparin (Polfia, Warsaw, Poland) to prevent clotting at the tip of the catheter. Throughout the experiment, the animals received intravenous infusion of normal saline (150 mN NaCl solution; 200 µL bolus and sustained infusion at 40 µL/min) containing 185 kBq/mL [3H]-inulin (PerkinElmer). After 30 min, the urinary bladder was exposed through an abdominal incision and catheterized for urine collection. The rats were allowed to stabilize for 60 min following the surgical procedure.

Experimental protocol. After the 60-min recovery period following surgery, two 30-min clearance periods were collected in each experiment. A control vehicle clearance period was taken during intravenous infusion of normal saline (40 µL/min). Then, P2X receptor agonist (α,β-methylene ATP; Sigma–Aldrich Chemical Co., Milwaukee, WI) was infused (as a bolus of 2 µmol/kg body weight in 200 µL over 2 min and as a continued infusion of 20 nmol/min/kg body weight), followed by a continuous infusion of normal saline or PPADS (Sigma–Aldrich Chemical Co.) of 70 µg/min/kg body weight (dissolved in normal saline). The second clearance period began 10 min after starting infusion of testing agents. Urine samples were collected for analysis of sodium and inulin excretion. During each clearance period, 100 µL blood was withdrawn from the artery for analyses of plasma sodium and inulin concentrations. At the end of the experiments, animals were euthanized by an overdose of thiopental. The clearance studies protocol is presented in Figure 1.
Denervation and tubular response to P2X agonists

Experimental groups. Group 1: effect of systemic infusion of P2X receptor agonists (αβ-meATP and βγ-meATP) on renal function in Sham rats.

One hour after initiation of the normal saline infusion, the first 30-min control clearance (vehicle) was taken. Ten minutes after the ATP analogue bolus injection, the second clearance period began, during which the rats were infused (bolus and continuous infusion, see above) with either αβ-meATP (n = 7), βγ-meATP (n = 6) or normal saline vehicle (n = 5).

Group 2: effect of systemic infusion of P2X receptor agonists, αβ-meATP (n = 6) and βγ-meATP (n = 6), and normal saline (n = 5) on renal function in DNx rats.

The experimental protocol was the same as in Group 1, but with DNx rats.

Group 3: effect of systemic co-infusion of P2X receptor agonists (αβ-meATP and βγ-meATP) with P2X receptor antagonist (PPADS) on renal function in Sham rats.

After the first 30-min control clearance (vehicle), PPADS was continuously infused (see above). Ten minutes after the ATP analogue bolus injection and initiation of the PPADS infusion, a second clearance period was started, during which the rats were infused (bolus and continuous infusion, see above) with either αβ-meATP, βγ-meATP or normal saline vehicle (n = 5 for each group).

Group 4: effect of systemic co-infusion of P2X receptor agonists (αβ-meATP and βγ-meATP) and normal saline (vehicle, n = 4) with P2X receptor antagonist (PPADS) on renal function in DNx rats.

The experimental protocol was the same as in Group 3, but with DNx rats (n = 5 for each group).

Analytic methods

Urine volume was determined gravimetrically. The glomerular filtration rate (GFR) was calculated based on the clearance of [3H]-inulin. [3H]-inulin activities in the plasma and urine were determined by liquid phase scintillation counting (1409 Wallac; LKB, Finland). The sodium concentrations in the plasma and urine were measured by flame photometry (Eppendorf, Germany). Fractional excretions of sodium (FFNa) and water (FEV) were derived from the ratio of sodium or water clearance values to inulin clearance.

Statistical analysis

Data are presented as mean ± SE. Statistical analysis was performed using a two-way analysis of variance followed by Dunnett’s multiple comparison test or unpaired t-test, as indicated. P-values < 0.05 or 0.01 were considered statistically significant.

Results

In normal intact rats (10 days before renal denervation; body weight: 239 ± 6 g), the daily excretions of urine and sodium were 4.6 ± 0.2 mL/100 g body weight and 0.62 ± 0.04 mmol/100 g body weight, respectively. Ten days after renal denervation (DNx; body weight: 295 ± 11 g), daily excretions of urine (4.9 ± 0.8 mL/100 g body weight) and sodium (0.56 ± 0.06 mmol/100 g body weight) were not significantly different (Table 1).

Table 1. Metabolic balance studies of sodium, potassium and urine excretions in rats after chronic renal denervation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before DNx</th>
<th>After DNx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>239 ± 6</td>
<td>295 ± 11*</td>
</tr>
<tr>
<td>Water intake, mL/24 h/100 g b.w.</td>
<td>10.2 ± 1.4</td>
<td>9.3 ± 0.7</td>
</tr>
<tr>
<td>Urine volume, mL/24 h/100 g b.w.</td>
<td>4.6 ± 0.2</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Urine specific gravity, g/L</td>
<td>1017 ± 1</td>
<td>1015 ± 1</td>
</tr>
<tr>
<td>Sodium excretion, mmol/24 h/100 g b.w.</td>
<td>0.62 ± 0.04</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>Potassium excretion, mmol/24 h/100 g b.w.</td>
<td>1.70 ± 0.05</td>
<td>1.44 ± 0.11</td>
</tr>
</tbody>
</table>

*aValues are means ± SE. b.w., body weight.

*p < 0.05 before versus after DNx.

The per cent changes of the mean FFNa values induced by αβ-meATP were significantly different (P < 0.05) in DNx rats and Sham rats (166 and 114%, respectively). Systemic infusion of saline (vehicle) and time-vehicle control did not change renal function with respect to GFR, urine volume or sodium excretion, in either group. In both Sham and DNx, systemic infusion of PPADS to block P2X receptors had no effect on FFNa and FEV and combined administration of PPADS and αβ-meATP or PPADS and βγ-meATP did not significantly increase FENa and FEV (Figures 2 and 3). MAP and GFR did not change during any clearance periods.

To measure Na/K-ATPase activity and study its regulation by αβ-meATP and βγ-meATP, we analysed the changes of ouabain-sensitive ATPase activity in proximal tubules isolated from innervated (Con) and denervated kidney (DNx). The results are presented in Table 3. In both groups, ouabain-sensitive ATPase activity represented 38% of total ATPase activity. Similar basal Na/K-ATPase activities were observed in DNx and Con (375 ± 18 versus 356 ± 19 nmol/min/mg protein). Both αβ-meATP and βγ-meATP (10−6 M) decreased Na/K-ATPase activity, with maximal inhibition of 20% (P < 0.05) in Con and DNx. These effects were blocked in the presence of PPADS, a P2 receptor antagonist. Ouabain-insensitive ATPase activity was not affected by αβ-meATP or βγ-meATP at 10−6 M.

Discussion

Changes in renal sympathetic nerve activity play a significant role in controlling body fluid homeostasis during normal daily activity and in the pathophysiology of many clinical conditions. Renal nerves end in close proximity to the epithelial cells of proximal tubules and the thick ascending limb of the loop of Henle. These adrenergic endings use ATP as a co-transmitter of NE and can directly increase tubular sodium reabsorption in these parts of nephron [1, 2]. Our previous study showed that P2X receptor activation leads to increased sodium excretion in the urine of anaesthetized rats, at

In the present study, reduction of sympathetic activity via renal sympathetic denervation resulted in improved natriuretic effects of systemically applied P2X receptor agonists, α,β-methyleneATP and β,γ-methyleneATP. The model of chronic bilateral surgical kidney denervation used in the present study is comparable to the catheter-based denervation by low-power radiofrequency; both are characterized by a several-fold decrease of kidney NE content or kidney NE spillover, reduction of renin activity and renal function, as measured by serum creatinine-based estimation of GFR, remains unchanged [1, 2, 5, 6].

The substantially improved sodium and water excretion in urine in response to renal denervation is likely due to combined beneficial effects of sympatheoinhibition and reduced NE release on regional tubular and direct cellular activity. It is generally accepted that renal sodium excretion is controlled primarily by mechanisms sensitive to changes in body fluid volume including the renal nerves activities. This action is crucial for sodium and water homoeostasis. Removal of the neural input by renal denervation and subsequent natriuresis may be expected to be compensated by various mechanisms including supersensitivity to circulation catecholamines, intrarenal compensatory mechanism or activity of renin–angiotensin–aldosterone system. The compensation mechanisms are probably responsible for no significant differences in sodium and water excretion before and after kidney denervation. This may also be due to low baseline renal sympathetic activity in conscious rats. However, the published literature suggests that changes of sympathetic nerves activity are imperative for renal adaptation to changes in sodium balance; renal denervation results in an inability of the kidney to appropriately lower urinary sodium excretion during dietary sodium restriction, which leads to

![Fig. 2. Changes of fractional sodium excretion (ΔFFNa) with systemic infusion of P2X receptor agonists (α,β-methyleneATP and β,γ-methyleneATP) in the absence and presence of P2X receptor blockade (PPADS) in sham-operated (Sham) and renal denervated (DNx) rats. *P < 0.01 Sham versus DNx (unpaired t-test).](https://academic.oup.com/ndt/article-abstract/27/9/3443/1858188)

![Fig. 3. Changes of fractional urine excretion (ΔFFV) with systemic infusion of P2X receptor agonists (α,β-methyleneATP and β,γ-methyleneATP) in the absence and presence of P2X receptor blockade (PPADS) in sham-operated (Sham) and renal denervated (DNx) rats. *P < 0.01 Sham versus DNx (unpaired t-test).](https://academic.oup.com/ndt/article-abstract/27/9/3443/1858188)
an increase in sympathetic activity [1]. The role of sympathetic nerves is profoundly expressed in anesthetized rats in clearance experiments. We and others have shown that sodium and water excretion increase after kidney denervation due to a decrease in sodium reabsorption in the proximal tubule [2].

In our experiments, the natriuretic effect was induced by methylene ATP analogues, α,β-methyleneATP and β,γ-methyleneATP, that are relatively resistant to nucleotide phosphohydrolases. Pharmacological and biochemical studies have shown that these agonists are equipotent at P2X1; however, α,β-methyleneATP is much more potent at P2X1 and β,γ-methyleneATP at P2X2,4,5,7 [19]. Importantly, both analogues have no appreciable potency at P2Y receptors [20]. The natriuretic effects of α,β-methyleneATP and β,γ-methyleneATP were abolished by PPADS, a non-selective P2 receptor antagonist (IC50 1–2.6 μM at P2X1,2,3,5) [21]. These findings were in accordance with previous experiments demonstrating the expression of P2X1,4,5,6 receptors in established proximal renal cell lines [8, 22]. Proximal tubule sodium reabsorption may be modulated by activity of the adrenergic system where ATP is a co-transmitter, likely via stimulation of renal nerves and activation of P2 receptors in adrenergic nerves termini [23]. However, activation of extranervous P2 receptors decreases proximal tubule sodium reabsorption. Our present observations suggest interplay between the adrenergic and purinergic systems in the kidney. In vitro and in vivo data from previous studies have demonstrated the involvement of P2X1,2,3,7 receptor expression in trauma/axotomy and suggest a contribution of this mechanism in the development of purinergic sensitivity after chronic axotomy [24].

On the other hand, pharmacological sympathectomized rats exhibit a significantly enhanced effect of NE on inositol phosphate production in renal glomerular cells, whereas ATP-induced inositol phosphate production is unaffected [25]. This suggests that regulation of adrenergic and purinergic signalling may occur independently.

In the present study, α,β-methyleneATP and β,γ-methyleneATP decreased Na/K-ATPase activity in isolated rat proximal tubules by an average of 20%, and this effect was abolished by the P2 receptor antagonist PPADS. These observations are consistent with the hypothesis that stimulation of P2X receptors inhibits Na/K-ATPase activity and sodium transport by proximal tubule cells [26]. Interestingly, similar levels of Na/K-ATPase inhibition were induced by α,β-methyleneATP and β,γ-methyleneATP in proximal tubules isolated from innervated and denervated kidneys, suggesting that the increased natriuretic response of denervated kidney is probably not related to changes in Na/K-ATPase activity. Other molecular targets in renal proximal tubule, including the sodium/proton exchanger (isoform NHE3), should be investigated in further studies.

The renal effect of extracellular nucleotides during systemic infusion into animals may result from P2 receptor activation in the kidney and other organs, including the central nervous system. It has been previously shown that extracellular ATP induces dopamine release in the rat striatum [27]. Dopamine is a potent natriuretic factor affecting Na/K-ATPase activity, but the present investigation did not determine the central mechanism of P2 receptor activation. Additionally, the kidney is a source of activating signals, which communicate with central autonomic pathways via the afferent renal nerves and, in turn, influence sympathetic output to a variety of vascular beds and modulate the release of neurohypophyseal hormones [28, 29]. Recent studies have shown that functional renal denervation, targeting both effenter sympatheitic nerves and afferent sensory nerves, has potential as a novel therapeutic strategy in resistant hypertension [5, 6]. Furthermore, renal denervation occurs in kidney transplantation as an inherent consequence of the surgery, and this direct consequence of this procedure may be important to consider in nephrology patients. Our present study sheds light on the potential involvement of purinergic signalling in maintenance of sodium homeostasis after sympathetic inhibition in these patients. In view of the present data, it is plausible that P2X receptor activation may contribute to the correction of blood pressure. It is important to note that P2X receptors are under ongoing stimulation because of basal nucleotide release from cells and additional stimulation of ATP release; neurotransmission, inflammation and platelet aggregation may increase the activation level and affect renal sodium reabsorption [30, 31].

Based on our findings, we propose that adrenergic activity influences the renal tubular response to P2X receptors activation. Understanding the role of adrenergic tone in the renal action of P2 receptor agonists is critical to understanding renal physiology as well as the development of tubular dysfunction after kidney transplantation or renal sympathetic nerve ablation in hypertension treatment. In this regard, the results of the present study are of both physiological and pathophysiological/clinical significance.

**Table 3.** Effect of P2X receptor agonists, α,β-methyleneATP and β,γ-methyleneATP, on ATPase activity in renal proximal tubules of control (Con) and renal denervation rats (DNx)

<table>
<thead>
<tr>
<th>Groups</th>
<th>ATPase (nmol/min/mg protein)</th>
<th>Total</th>
<th>Ouabain insensitive</th>
<th>Ouabain sensitive</th>
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<tr>
<td>Vehicle (n = 5)</td>
<td>927 ± 36</td>
<td>576 ± 26</td>
<td>356 ± 19</td>
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<tr>
<td>α,β-methyleneATP</td>
<td>839 ± 21*</td>
<td>548 ± 14</td>
<td>290 ± 13*</td>
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<tr>
<td>β,γ-methyleneATP</td>
<td>840 ± 27**</td>
<td>555 ± 17</td>
<td>285 ± 22**</td>
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<tr>
<td>PPADS + α,β-methyleneATP</td>
<td>906 ± 32</td>
<td>565 ± 25</td>
<td>341 ± 25</td>
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<tr>
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<td>916 ± 23</td>
<td>597 ± 19</td>
<td>320 ± 23</td>
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<tr>
<td>PPADS</td>
<td>887 ± 41</td>
<td>561 ± 17</td>
<td>338 ± 33</td>
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<th>Groups</th>
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<td>n = 5</td>
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<td>927 ± 36</td>
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<tr>
<td>n = 5</td>
<td>595 ± 22</td>
<td>598 ± 20</td>
<td>617 ± 13</td>
<td>604 ± 11</td>
<td>602 ± 14</td>
<td>652 ± 23</td>
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<td>n = 5</td>
<td>375 ± 18</td>
<td>301 ± 24*</td>
<td>298 ± 35*</td>
<td>351 ± 17</td>
<td>379 ± 22</td>
<td>352 ± 24</td>
<td>375 ± 18</td>
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*Values are means ± SE; n, number animals.

**Conflict of interest statement.** None declared.

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References